Docket No.: 21581-00256-US1

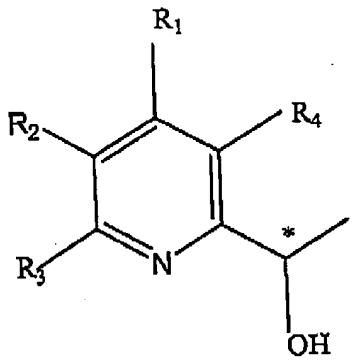
Application No. 10/669,503
Amendment dated
Reply to Office Action of July 12, 2006

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior listings and versions of the claims in this application.

Please cancel claim 15 without prejudice or disclaimer.

1. (Withdrawn-Currently Amended) A method of producing an optically active pyridineethanol derivative represented by the general formula

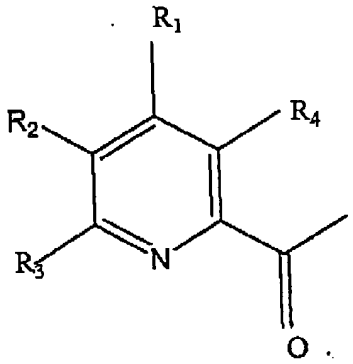


[2]

wherein R_1 and R_2 are bound to each other to form a 5- to 8-membered monocyclic heterocycle containing at least one hetero atom selected from the group consisting of oxygen, sulfur and nitrogen atoms, which heterocycle may optionally have a substituent(s), or a polycyclic heterocycle resulting from the condensation of such monocyclic heterocycle with another ring, which polycyclic heterocycle may optionally have a substituent(s),

R_3 and R_4 are the same or different and each represents a hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), or an alkoxy group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), and * indicates that the asterisked carbon atom is an asymmetric one,

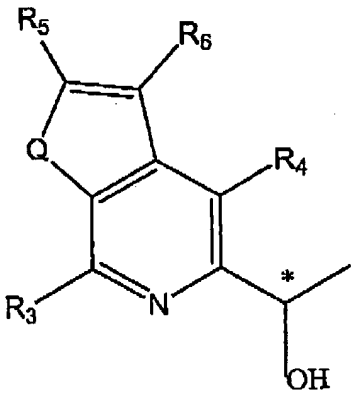
which method comprises stereoselectively reducing an acetylpyridine derivative represented by the general formula [1]:



[1]

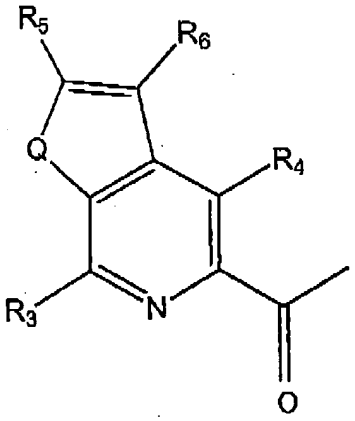
wherein R_1, R_2, R_3 and R_4 are as defined above, by causing an isolated enzyme or isolated enzyme source capable of asymmetrically reducing the same to act thereon.

2. (Withdrawn- Currently Amended) A method of producing an optically active pyridineethanol derivative represented by the general formula [4]:



[4]

wherein Q represents an oxygen or sulfur atom or a group of the general formula -N(D)-, in which N is a nitrogen atom and D represents a hydrogen atom or a monovalent protective group, R_3, R_4, R_5 and R_6 are the same or different and each represents a hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), or an alkoxy group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), and * indicates that the asterisked carbon atom is an asymmetric one, which method comprises stereoselectively reducing an acetylpyridine derivative represented by the general formula [3]:



[3]

wherein Q, R_3, R_4, R_5 and R_6 are as defined above, by causing an isolated enzyme or isolated enzyme source capable of asymmetrically reducing the same to act thereon.

3. (Withdrawn) The production method according to Claim 2, wherein Q is an oxygen atom.

4. (Withdrawn) The production method according to Claim 2, wherein Q is an oxygen atom,

R_3 is a hydrogen atom or a chlorine atom,

R_4 is a hydrogen atom,

R_5 is a hydrogen atom

and R_6 is a hydrogen atom or a methyl group.

5. (Withdrawn) The production method according to Claim 2, wherein Q is an oxygen atom and R_3, R_4, R_5 and R_6 each is a hydrogen atom.

6. (Withdrawn- Currently Amended) The production method according to Claim 1, wherein the reaction is carried out in the presence of an isolated enzyme capable of reducing the oxidized form nicotinamide adenine dinucleotide and/or the oxidized form nicotinamide adenine

dinucleotide phosphate to the respective reduced forms as well as a substrate for the reduction.

7. (Withdrawn- Currently Amended) The production method according to Claim 6, wherein said isolated enzyme for reduction to the reduced form is glucose dehydrogenase and said substrate for reduction is glucose.

8. (Withdrawn- Currently Amended) The production method according to Claim 6, wherein said isolated enzyme for reduction to the reduced form is formate dehydrogenase and said substrate for reduction is formic acid.

9. (Withdrawn- Currently Amended) The production method according to Claim 1, wherein said isolated enzyme or isolated enzyme source is derived from a microorganism selected from the group consisting of microorganisms of the genera *Ashbya, Candida, Cryptococcus, Clavispora, Debaryomyces, Dipodascus, Galactomyces, Geotrichum, Guilliermondella, Hanseniaspora, Hansenula, Hyphopichia, Issatchenkia, Kluyveromyces, Kuraishia, Lodderomyces, Metschnikowia, Ogataea, Pachysolen, Pichia, Rhodosporidium, Rhodotorula, Saccharomycopsis, Schwanniomyces, Sporidiobolus, Sporobolomyces, Schizoblastosporion, Stephanoascus, Torulaspora, Trigonopsis, Trichosporon, Willopsis, Yamadazyma, Zygosaccharomyces, Alcaligenes, Bacillus, Brevibacterium, Cellulomonas, Corynebacterium, Jensenia, Ochrobactrum, Pseudomonas, Rhodococcus* and *Tsukamurella.*

10. (Withdrawn- Currently Amended) The production method according to Claim 9, wherein the product optically active pyridineethanol derivative has the S absolute configuration and said isolated enzyme or isolated enzyme source is derived from a microorganism selected from the group consisting of microorganisms of the genera*Ashbya, Candida, Cryptococcus, Clavispora, Debaryomyces, Dipodascus, Galactomyces, Geotrichum, Guilliermondella, Hanseniaspora, Hansenula, Hyphopichia, Issatchenkia, Kluyveromyces, Kuraishia, Lodderomyces, Metschnikowia, Ogataea, Pachysolen, Pichia, Rhodosporidium, Rhodotorula, Saccharomycopsis, Schwanniomyces, Sporidiobolus, Sporobolomyces, Schizoblastosporion, Stephanoascus, Torulaspora, Trigonopsis, Trichosporon, Willopsis, Yamadazyma, Zygosaccharomyces, Alcaligenes, Bacillus, Brevibacterium, Cellulomonas,*

Corynebacterium, Jensenia, Ochrobactrum, Pseudomonas, Rhodococcus and *Tsukamurella.*

11. (Withdrawn- Currently Amended) The production method according to Claim 9, wherein the product optically active pyridineethanol derivative has the R absolute configuration and said isolated enzyme or isolated enzyme source is derived from a microorganism selected from the group consisting of microorganisms of the genera *Candida, Ogataea, Pichia, Yamadazyma, Brevibacterium,* and *Corynebacterium.*

12. (Currently Amended) An isolated enzyme having the following physical and chemical properties (1) to (3):

(1) Activity: It stereoselectively reduces 5-acetylfuro[2,3-c]pyridine, to 5-(1-(R)-hydroxyethyl)furo[2,3-c]pyridine, in the presence of reduced form nicotinamide adenine dinucleotide as a coenzyme~~, to give 5-(1-(R)- hydroxyethyl)furo[2,3-c]pyridine~~;

(2) Specificity: It has reducing ability against ketones and aldehydes but ~~is very low in~~ the reducing activity against carbocyclic ketones and the α-position keto group of α-keto acids is not more than 10% at 30°C and at pH 6.5, when the reducing activity against 5-acetylfuro[2,3-c]pyridine is taken as 100%; and

(3) Molecular weight: It shows a molecular weight of about 60,000 in gel filtration analysis and a molecular weight of about 29,000 in SDS polyacrylamide electrophoresis,

which is isolated from a microorganism belonging to the genus *Candida*.

13. (Currently Amended) The isolated enzyme according to Claim 12 which has the following physical and chemical properties (4) to (6):

(4) Optimal temperature: 50 °C to 55 °C;

(5) Optimal pH: 5.0 to 6.0; and

(6) Inhibitor: It is inhibited by the mercury ion.

14. (Currently Amended) An isolated enzyme specified below under (a) or (b):

(a) An isolated enzyme comprising ~~an~~ the amino acid sequence ~~shown under~~ of SEQ ID NO:1 in the sequence listing; or

(b) An isolated enzyme comprising an amino acid sequence ~~derived~~ obtained from the amino

acid sequence ~~shown under~~ of SEQ ID NO:1 ~~in the sequence listing~~ by deletion, substitution and/or addition of one ~~or several~~ amino acid ~~acids~~ and having an activity by which 5-acetylfuro[2,3-c]pyridine is stereoselectively reduced to 5-(1-(R)-hydroxyethyl)furo[2,3-c]pyridine.

15. (Canceled)

16. (Currently Amended) The isolated enzyme according to Claim 12 which is ~~derived~~ isolated from *Candida maris* ~~Candida maris~~.

17. (Currently Amended) The isolated enzyme according to Claim 12 which is ~~derived~~ isolated from *Candida maris* ~~Candida maris~~ IFO 10003.

18. (Withdrawn) The production method according to Claim 1, wherein said isolated enzyme is defined according to Claim 12

and the product optically active pyridineethanol derivative has the R absolute configuration.

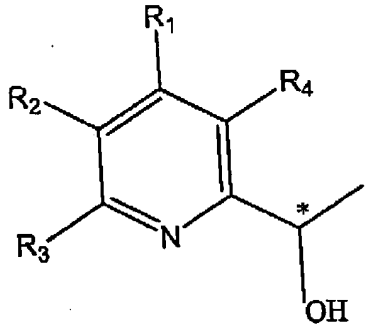
Claims 19-32 cancelled.

33. (Withdrawn- Currently Amended) The production method according to Claim 1, wherein said isolated enzyme is the transformant having the recombinant vector containing a DNA coding for an isolated enzyme specified below under (a) or (b):

(a) An isolated enzyme comprising an amino acid sequence shown under SEQ ID NO:1 in the sequence listing;

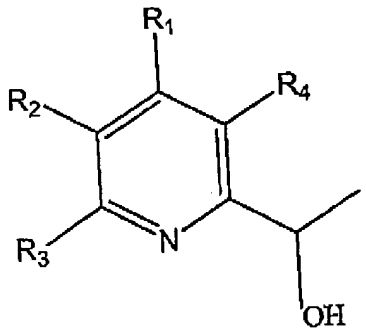
(b) An isolated enzyme comprising an amino acid sequence derived from the amino acid sequence shown under SEQ ID NO:1 in the sequence listing by deletion, substitution and/or addition of one or several amino acids and having an activity by which 5-acetylfuro[2,3-c]pyridine is stereoselectively reduced to 5-(1-(R)-hydroxyethyl)furo[2,3-c]pyridine, and said product optically active pyridineethanol derivative has the R absolute configuration.

34. (Withdrawn- Currently Amended) A method of producing an optically active pyridineethanol derivative having the S absolute configuration and represented by the general formula [6]:



[6]

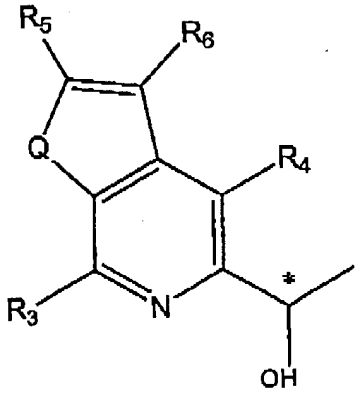
wherein R_1 and R_2 are bound to each other to form a 5- to 8-membered monocyclic heterocycle containing at least one hetero atom selected from the group consisting of oxygen, sulfur and nitrogen atoms, which heterocycle may optionally have a substituent(s), or a polycyclic heterocycle resulting from the condensation of such monocyclic heterocycle with another ring, which polycyclic heterocycle may optionally have a substituent(s),
and R_3 and R_4 are the same or different and each represents a hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), or an alkoxy group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), and * indicates that the asterisked carbon atom is an asymmetric one,
which method comprises causing the isolated enzyme according to any of Claims 12 to 17 and/or the transformant according to any of Claims 26 to 32 to act on a pyridineethanol derivative represented by the general formula [5]:



[5]

wherein R_1, R_2, R_3 and R_4 are as defined above, to thereby preferentially oxidize the R form of the pyridineethanol derivative
and recovering the remaining S form of the pyridineethanol derivative.

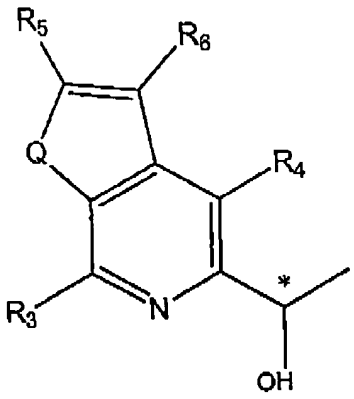
35. (Withdrawn- Currently Amended) A method of producing an optically active pyridineethanol derivative having the S absolute configuration and represented by the general formula [8]:



[8]

wherein Q represents an oxygen or sulfur atom or a group of the general formula -N(D)-, in which N is a nitrogen atom and D represents a hydrogen atom or a monovalent protective group, R_3, R_4, R_5 and R_6 are the same or different and each represents a hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), or an alkoxy group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), and * indicates that the asterisked carbon atom is an asymmetric one, which method comprises causing the isolated enzyme according to any of Claims 12 to 17 and/or

the transformant according to any of Claims 26 to 32 to act on a pyridineethanol derivative represented by the general formula [7]:



[7]

wherein Q, R_3, R_4, R_5 and R_6 are as defined above, to thereby preferentially oxidize the R form of the pyridineethanol derivative
and recovering the remaining S form of the pyridineethanol derivative.

36. (Withdrawn) The production method according to Claim 35, wherein Q is an oxygen atom.

37. (Withdrawn) The production method according to Claim 35, wherein Q is an oxygen atom,
R_3 is a hydrogen atom or a chlorine atom,
R_4 is a hydrogen atom,
R_5 is a hydrogen atom
and R_6 is a hydrogen atom.